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Bellarmine University, awelp01@bellarmine.edu

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The Making of a Pathogen: Implications of Phage Domestication in *Acinetobacter baumannii*

Allison Welp

April 28, 2017

A Senior Honors Thesis Presented Through the
Bellarmine University Honors Program

Under the Direction of Dr. Daniel Golemboski

Reader: Dr. Gail Ramirez-Icaza
Reader: Dr. Amanda Krzysiak

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The Making of a Pathogen: Impacts of Phage Domestication in *Acinetobacter baumannii*

Introduction

Populations of bacteria continue to change and new pathogens have consequently emerged. As bacterial pathogens evolve, an increasing number of virulence factors are acquired. One of the most significant changes in the bacterial population is due to the introduction of antibiotics and subsequent increase in antibiotic resistance. This problem poses a significant threat to human health. Until new antibiotics are created or novel therapeutic strategies are implemented, humans are rendered defenseless during infection. The species *Acinetobacter baumannii* has undergone significant changes and is now recognized as one of the most threatening pathogens due to its high antibiotic resistance profile. What was once an innocuous environmental organism is now one of the greatest clinical threats. With a growing prominence in healthcare, the evolution of *A. baumannii* is particularly unique; to better understand the rise to its present state, several clinical isolates of *A. baumannii* were analyzed to discern the changes that have occurred to contribute to its pathogenicity. Upon analysis, several intact and incomplete prophages were discovered that were contained within the bacterial chromosome. Remnants of these bacterial viruses have been shown to be advantageous in a number of other bacteria, but no relationship to *A. baumannii* has been previously described. In response to these changes, the conservation of bacteriophage genes was assessed in relation to the current increase in antibiotic resistance and virulence of *Acinetobacter baumannii.*

Bacteriophages are bacterial viruses that naturally infect bacteria, integrating their own genetic information into the bacterial genome and become prophages. By using bacteria as a host, prophages multiply with bacteria through replication. Through lysis of the bacterium, bacteriophages are removed from the bacterial genome, killing their host. Despite this parasitic relationship, the inclusion of bacteriophage genes within a genome has the potential to increase the virulence of the bacteria. Due to these beneficial changes in pathogenicity, bacteria have selected for these advantageous modifications. Some species have even maintained defective phages within their genome, corresponding to progressive increases in virulence across the bacterial population. Unlike other non-pathogenic *Acinetobacter* species, *A. baumannii* has maintained greater numbers of incomplete prophages within its genome, particularly that of *Acinetobacter* phage Bϕ-B1251. The acquisition and maintenance of defective phage elements appears to be increasing over time, suggesting that *A. baumannii* has undergone a directional evolution, selecting for the advantages bacteriophage genes confer.

I. PATHOGENICITY AND ANTIBIOTIC RESISTANCE

The coexistence of humans and microorganisms has historically been documented, accepted, and understood. But the human relationship with bacteria is not as straightforward as some believe. As we continue to elucidate the intricacies of our connectedness with bacteria, these organisms continue to change. Humans have a propensity to solely associate bacteria with infections; despite the bad reputation of bacteria, they also contribute to a number of beneficial human interactions. The human body contains a number of microbial environments, including the skin, respiratory tract, gastrointestinal tract, and conjunctiva of the eyes. In total, these environments contain approximately 10^{14} bacteria, ten times the number of human cells within the body (Davis, 1996). During birth, bacteria begin to colonize these sites. While the types of bacteria contained within the body continue to fluctuate, one thing remains the same: these bacteria are essential for health. By competing with pathogenic species for nutrients, the normal bacterial flora prevent the colonization of harmful bacteria. Others produce enzymes, such as proteases, peroxides, and bacteriocins capable of killing invading bacteria and denaturing harmful bacterial toxins (Quigley, 2013).

While bacteria certainly confer great health advantages, not all species are beneficial. The term “pathogens” is applied to these species that are capable of causing disease. Bacteria have been implicated as pathogens throughout the course of history; lethal plagues have decimated empires and resulted in the fall of rulers. The first recorded plague, the Great Pestilence of Egypt, occurred in 3180 BC and is documented in the Bible (Krause, 1992). Since this time, outbreaks of bacteria continue to be noted and have caused epidemics that unceasingly altered the course of history. The human background is characterized by these microbiological threats and outbreaks such as the Bubonic Plague, cholera, typhoid fever, diphtheria, leprosy, and

anthrax attacks. Not only have these events caused widespread fear and significant deaths, but they have also ushered in greater scientific understanding and have prompted the development of treatments and therapeutics.

Even among pathogens, some are more destructive than others. The degree of pathogenicity, the ability to cause disease, is termed virulence. Bacteria have developed a number of mechanisms to increase their pathogenicity. Broadly, two factors are analyzed to describe bacterial virulence: toxicity and invasiveness. Toxicity describes the degree of harm while invasiveness describes the ability to penetrate and spread throughout a host (Beceiro, Tomás, & Bou, 2013). These factors are essential in order to overcome the protective barriers of a host, including the populations of bacteria contained within a host. Nevertheless, host status is another critical factor that must be considered when discussing pathogenicity. Some organisms are only capable of infecting immunosuppressed individuals, those with weakened immune systems due to chronic illness, age, diet, and stress. Pathogens that are incapable of causing infection in healthy people, but do cause infection in immunocompromised individuals are termed “opportunistic” pathogens.

Despite the devastation associated with bacterial illness, the discovery of antibiotics promised a new era of protection from bacterial pathogens. When Alexander Fleming first discovered the effects of penicillin in 1928, the microbial world was transformed and a sense of hope emerged (Fleming, 1944). When Fleming noticed that a mold contaminant introduced into his cultures of *Staphylococcus aureus* killed his bacteria, he was prompted to begin studying this strange occurrence. It took many years for this compound to be successfully isolated before it was first used clinically in 1941 by Charles Fletcher (Fletcher, 1984). The antibiotic penicillin was first introduced into routine hospital procedures by the Mayo Clinic in 1943 in order to treat

patients with bacterial infections (Herrell, 1944). Since this time, hundreds of antibiotics have been developed. These antibiotics range in effectiveness, some treating specific classes of bacteria (narrow-spectrum) while others target many classes of bacteria (broad-spectrum).

Antibiotics have become an integral component to healthcare; without antibiotics, bacteria would be remarkably more devastating for humans. Before antibiotics were used as treatment, 30% of all deaths were attributable to bacterial infections (Fair & Tor, 2014). Today, this number is significantly lower. By reducing infections, hospital stays, and mortality rates, antibiotics are necessary to improve patient outcome. Despite their apparent promise, what once protected the health of millions is now not always a reliable treatment option. We have used antibiotics for many years and have reaped the benefits they provide in controlling disease. However, this reliance has yielded consequences; bacteria have been exposed to the same antibiotics for so long that they have adapted to these medications. The evasion and antagonistic ability bacteria have developed against antibiotics has been exacerbated by the acquisition of resistance mechanisms across bacterial populations. This alarming trend has been compounded by inappropriate antibiotic use in healthcare and agriculture (Demirjian, 2015).

With time and exposure to antibiotics, bacteria continue to acquire resistance at a disconcerting rate. When antibiotics are not used by the body—due to incorrect or unnecessary prescriptions—increasing amounts are released into the environment and to bacterial populations within the body. Not only do excess antibiotics often kill healthy, beneficial bacteria, they also select for strains of bacteria that are most resistant. When faced with antibiotics, only those strains harboring resistance genes or possessing beneficial mutations are able to survive. These bacteria proliferate and take the place of susceptible bacteria, expanding the resistant population (Bearson & Brunelle, 2015). Increasing amounts of resistant bacteria also lead to increased

opportunities to transfer these genes to other bacteria in the environment, as resistance genes can be transmitted to other organisms through horizontal gene transfer.

Antibiotic resistance has become one of the most serious threats of our time, but society and some healthcare workers often overlook its prevalence. Every year approximately two million people become infected with bacteria that are resistant to antibiotics; of these infections, about 23,000 people die as a direct result of infection (Demirjian, 2015). When bacteria develop resistance to some antibiotics, we must turn to other antibiotics. This progression continues until bacteria ultimately become resistant to all antibiotics. Because no approved alternative options exist for treatment of bacterial infections, we are left defenseless.

Unfortunately, antibiotic-resistant organisms have adapted and acquired mechanisms that allow for survival, counteracting the mechanisms antibiotics use to kill or inhibit bacterial growth. These organisms have undoubtedly become one of the largest threats to healthcare by becoming progressively more difficult to treat. Because these resistant organisms are not easily eradicated, they can also be rapidly spread throughout hospitals. These problems impact every aspect of healthcare; approximately $20 billion of excess is spent to treat antibiotic-resistant infections (Fair & Tor, 2014). On average in the United States, patients suffering from these infections spend a total of 8 million extra days in the hospital and antibiotics comprise over 30% of pharmacy budgets (Fair & Tor, 2014).

II. BACTERIOPHAGES

Just as humans become infected with bacteria and viruses, bacteria are also face natural predators in the form of bacterial viruses: bacteriophages. These viral particles exploit bacterial cells for growth, integrating their own genetic information into the genome of bacteria. Once the

bacteriophage genetic information has been incorporated into the bacterial genome, this genetic material is termed a "prophage." These viruses may be lytic, quickly killing their host after replication, or they may remain lysogenic, entering a period of dormancy. During the lysogenic cycle, phage DNA is integrated within bacterial chromosomes, replicating alongside host DNA (Fortier & Sekulovic, 2013).

Despite their destructive nature, both bacteriophages and bacteria confer some natural benefit to one another. Bacteriophages profit from promoting the survival and proliferation of their bacterial hosts; when bacteria are killed, the phages they contain are also harmed. Bacteriophages rely on the health and replication of their hosts in order to produce more viral particles. During lysogeny, bacteria often acquire enhanced fitness through increased virulence, pathogenicity, and stress tolerance (Fortier & Sekulovic, 2013). Due to these advantageous changes, the host is protected against further phage infection and phagocytosis. Bacteriophages also encode accessory genes that increase host fitness; these include increased growth under nutrient limitation, biofilm formation, and antibiotic tolerance. Some prophages also encode regulatory switches, influencing gene expression (Bobay, Touchon, & Rocha, 2014).

In addition, bacteriophages also increase horizontal gene flow throughout the entire bacterial population, allowing genetic information to be more easily transferred between organisms (Chen, Golding, Sawai, Guo, & Cox, 2005). Because bacteriophages are the most abundant organisms on Earth—ten times more abundant than bacteria—they harbor a substantial amount of genetic information. Approximately 20% of all bacterial genomes are derived from bacteriophage DNA. Of this information, a large amount confers resistance to antibiotics, enhances conjugation and biofilm formation, and also helps to endure a number of environmental stresses (Wang et al., 2010). Bacteriophages are the single largest contributor to horizontal

(lateral) gene transfer—transfer of genetic information between two organisms. Therefore, bacteriophages promote bacterial diversity; due to the presence and position of prophages or phage-like elements, virtually every strain of bacteria is unique. Almost every organism has been found to contain prophages or harbor genes derived from bacteriophages (Wang et al., 2010).

In order to sustain this massive population, it is necessary for phages to ensure their own survival and proliferation; this could not be done without their bacterial hosts. If bacteriophages were solely harmful, bacteria would slowly adapt to resist phage attack. Because phages do often benefit their host, bacteria continue to select for the advantageous features that prophages provide (Feiner et al., 2015). In this manner, bacteria maintain these favorable traits and "domesticate" their prophages. However, these domesticated elements are often removed from the bacterial genome when they are replaced by similar traits carried by new prophages. Therefore, the bacterial genome remains in a state of continuously shifting state of acquisition and loss of phage-derived genes (Bobay et al., 2014).

Bacteriophages represent a major vector of bacterial genetic diversity, creating increasingly harmful pathogens. A number of studies utilizing genome mining and comparative genomics have demonstrated the pervasiveness of prophages in the majority of bacterial genomes. Not only have prophages impacted the divergence of different bacterial species, but they also account for significant intra-species variation in pathogenicity and virulence (Wang et al., 2010). The virulence of many species of bacteria—including *Escherichia coli, Streptococcus pyogenes, Salmonella enterica,* and *Staphylococcus aureus*—is due to acquisition of prophages and phage genes (Fortier & Sekulovic, 2013). *Escherichia coli* strain O157:H7, the causative agent of entero-hemorrhagic food poisoning, is especially virulent due to the acquisition of two

prophages. These two prophages, Sp5 and Sp15, both encode Shiga-toxin production in *E. coli* O157:H7, resulting in the devastating virulence of this pathogen (Fortier & Sekulovic, 2013). Likewise, *Vibrio cholerae*, the causative agent of cholera, is only able to secrete cholera toxin (CT) due to the inclusion of filamentous phage CTXϕ within its genome. When pandemic strains of *V. cholerae* were compared to environmental non-toxigenic El Tor strains of *V. cholerae*, it was discovered that the El Tor strain lacked the CTXϕ prophage (Canchaya, Fournous, & Brussow, 2004). Prophages have also been demonstrated to have similar effects on other organisms, including *Corynebacterium diptheriae, Clostridium botulinum, Staphylococcus aureus,* and others (Chen et al., 2005). Phage genes have been found to encode toxins, effector proteins, and enzymes including superoxide dismutase, staphylokinase, phospholipase, adhesion factors, and proteins involved in resistance (Fortier & Sekulovic, 2013). Therefore, the presence of prophages within a bacterial genome certainly does have a profound effect of the virulence of an organism, even among the same species.

Through time, prophages may not remain active and capable of lysis. In fact, a number of prophages are impacted by mutations that occur in bacterial genomes. When segments of the prophage are deleted or only certain genes are maintained through replication, a prophage becomes inactive—referred to as a "cryptic" prophage. Despite their incomplete nature, these phage remnants still impact the pathogenicity and virulence of their host. However, these events may not be random occurrences. Louis-Marie Bobay, Marie Touchon, and Eduardo Rocha introduced the concept of phage domestication in 2014. In this study, the dynamics of prophage degradation were analyzed during vertical gene transfer (from parent to offspring). It was found that certain phage sequences were maintained throughout replicative events, including phage sequences coding for certain phage functions, particularly tail and lysis proteins (Bobay et al.,

2014). This study concluded that phage domestication was adaptive to individual bacteria, but advantageous additions were maintained by purifying selection (Bobay et al., 2014).

A number of virulence mechanisms have been discovered to result from the maintenance of incomplete prophages in bacterial genomes; these conserved genes can still produce phage killer particles and R/F-type bacteriocins that are capable of killing sensitive cells. Cryptic prophages may still be capable of forming transducing particles, phages carrying host DNA in place of their own, as well as gene transfer agents (GTAs) that allow for exchange of genetic material between bacteria. Also, incomplete prophages present in the genome may interrupt assembly of other phages or bacterial functions (Bobay et al., 2014).

The impact of cryptic prophages on pathogenicity can be demonstrated by analyzing the genome of *Escherichia coli.* This well-known species of bacteria is significantly different than its closest relative, *Salmonella.* In particular, the K-12 strain of *E. coli* has gained 18% of its genetic information since it diverged from *Salmonella* 100 million years ago (Wang et al., 2010). This novel information is primarily due to bacteriophages. K-12 contains one complete Lambda prophage and nine cryptic prophages. In a previous study, when all of these prophage elements were deleted, this strain became susceptible to one half of the normal minimum inhibitory concentration (MIC) of quinolone and β-lactam antibiotics, and its ability to form biofilms was greatly diminished. In addition, this modified strain displayed lower rates of growth in nutrient-poor and nutrient-rich media as well as decreased temperatures. Therefore, cryptic phage elements also confer increased resistance to antibiotics and enhanced growth in stressful environments (Wang et al., 2010).

III. ANTIBIOTIC RESISTANCE: CURRENT THREATS

Monitoring of antibiotic resistance potential is essential in dictating treatment solutions and observing the evolution of bacteria. The Centers for Disease Control (CDC) has published guidelines defining "Urgent," "Serious," and "Concerning" threats. There are currently eighteen organisms identified and categorized by the CDC. Of these three classifications, three species of bacteria receive the highest level of public health attention: urgent. These bacteria are either causing severe outbreaks or have high potential of causing outbreaks. Immediate public health intervention must be taken to limit transmission of these species, as they are difficult to prevent and increasingly difficult to treat. These three antibiotic-resistant species are *Clostridium difficile*, carbapenem-resistant Enterobacteriaceae (CRE), and *Neisseria gonorrheae* (Prevention, 2013).

Unlike urgent threats, serious threats will not immediately become public health threats, but are expected to worsen to become urgent with declining availability of antibiotic options. These threats include: multidrug-resistant *Acinetobacter,* drug-resistant *Campylobacter,* fluconazole-resistant *Candida,* extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs), vancomycin-resistant *Enterococcus* (VRE)*,* multidrug-resistant *Pseudomonas aeruginosa,* drug-resistant non-typhoidal *Salmonella,* drug-resistant *Salmonella* typhi, drug-resistant *Shigella,* methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae,* and multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (Prevention, 2013).

Bacteria listed in the "concerning" category cause serious illness; while these organisms are resistant to a number of drugs, they remain sensitive to many therapeutic options. Once the functionality of these drugs are jeopardized, these organisms could become urgent threats. This

group includes vancomycin-resistant *Staphylococcus aureus,* erythromycin-resistant Group A *Streptococcus,* and clindamycin-resistant Group B *Streptococcus (Prevention, 2013).*

The World Health Organization also warns of the dangers of antibiotic resistance. They characterize antibiotic resistance as one of the three most pressing problems impacting human health. The acronym ESKAPE has been developed to warn of six of the most serious multi-drug resistant organisms threatening healthcare: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp. (Howard, O'Donoghue, Feeney, & Sleator, 2012). These species of bacteria comprise the causes of the majority of nosocomial (hospital-acquired) infections and are particularly difficult to control within the hospital setting.

IV. THE EMERGENCE OF *ACINETOBACTER BAUMANNII*

Within the span of a few years, *Acinetobacter baumannii* has become ubiquitous in healthcare, capable of causing a number of infections. Recognized as a "serious" threat by the Centers for Disease Control, this species of bacteria had not become widely recognized until recently. This species is an opportunistic pathogen, infecting critically ill and immunocompromised individuals, including newborns, elderly, pregnant women, and other diseased individuals. *A. baumannii* is characterized as a strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative Gram-negative cocco-bacillus (Howard et al., 2012). It is not a member of the normal flora of the skin or other human environments, meaning that it is considered a true pathogen when isolated from human sources.

A. baumannii is capable of causing a number of infections, often targeting moist tissue, such as mucous membranes (Howard et al., 2012). While *A. baumannii* most commonly causes community- and hospital-acquired pneumonia, it is also a notable cause of meningitis, bacteremia, wound infections, and urinary tract infections, with minor implications in other infections. Due to its unique ability to form biofilms, it is capable of colonizing the surface of the lungs, endotracheal tubes, urinary tract, and the eyes, resisting extraction by mechanical defenses such as coughing, urination, and blinking (Peleg, Seifert, & Paterson, 2008). Biofilms are colonies of bacterial that aggregate together and form a community physically held together by an extracellular polymeric substance (EPS) matrix (Donlan, 2002). Biofilms require exposed surfaces for attachment, but once formed, become sturdy masses of bacteria that resist mechanical removal and chemical destruction, including death by antibiotics. The formation of biofilms makes this pathogen especially threatening in a hospital environment, as it can colonize all surfaces in hospitals including counters, ventilation tubes, and catheters.

The presence of *A. baumannii* is also felt within our own community. *A. baumannii* is a frequently encountered pathogen in hospitals in Louisville, Kentucky, and has been notable for many years. The occurrence of this pathogen was analyzed at one single hospital in Louisville beginning in 2010. Considering the number of hospitals found in this one city, the impact of *A. baumannii* is actually much greater in this community. While it is associated with a number of varying infections at one specific hospital, it is most commonly associated with respiratory and wound infections (Table 1). Unfortunately, this data does not reflect the increasing rates of multi-drug resistant *Acinetobacter* in this hospital. Although the numbers of infections do not change significantly each year, based on this data, it is likely that a greater percentage of those isolated in 2016 are multi-drug resistant than the percentage seen in 2010.

The first strain of *Acinetobacter* was isolated from the soil in 1911, originally named *Micrococcus calco-aceticus.* The name of this organism was officially changed in 1971 to *Acinetobacter,* and today this genome comprises 48 species. Members of this genus are commonly isolated in the environment in soil and water, with the exception of *A. baumannii.* The species *A. baumannii* has yet to be isolated in the environment; rather, it is routinely isolated in hospital settings as a pathogen (Howard et al., 2012). The genus *Acinetobacter* remained largely innocuous throughout history, only isolated from the environment; the emergence of *A. baumannii* as a pathogen in the past few decades symbolized a significant shift. The first clinical account of *A. baumannii* was published by Schaub and Hauber in "A Biochemical and Serological Study of a Group of Identical Unidentifiable Gram-negative Bacilli from Human Sources" in 1948. The organisms in this study were originally identified as *Neisseria* species until it was noted that they failed to reduce nitrates. Rather than recognizing these species to be *A. baumannii* at this time, these isolates were referred to as *Bacterium antinatratum* and later identified to be a pathogenic *Acinetobacter* species (Schaub & Hauber, 1948).

Since 1948, this species has become increasingly more recognized in healthcare. In an article written in 1996 by Bergogne-Bérézin and Towner, *A. baumannii* was still described as a "relatively low-grade pathogen" (Bergogne-Bérézin & Towner, 1996). However, this pathogen has been gaining much more respect due to its successful virulence mechanisms and extensive antibiotic resistance profile, resulting primarily from the high degree of plasticity of its genome. In one study, significant differences were found in two *A. baumannii* clinical isolates taken from the same patient one week apart (Hornsey et al., 2011). One strain was taken prior to the patient beginning tigecycline therapy, while the other was obtained one week afterwards. During a single week, this organism accumulated a number of mutations within its genome that conferred

resistance to many antibiotics, including tigecycline (Hornsey et al., 2011). Studies such as this demonstrate the rapid evolution that *A. baumannii* is able to undergo in a very short time.

In the past, clinical isolates of *A. baumannii* were successfully treated with aminoglycosides, β-lactams, quinolones, and tetracyclines including genatmicin, minocycline, nalidixic acid, ampicillin, or carbenicillin; these antibiotics were successful in treating infections until resistance to these antibiotics was noted around 1975 (Bergogne-Bérézin & Towner, 1996). According to an article written by Bergogne-Bérézin and Towner in 1996, carbapenems such as imipenem and meropenem were the therapies of choice for *A. baumannii* infections. At this time, carbapenems were effective on “100% of strains” (Bergogne-Bérézin & Towner, 1996). However, the same is not true today; in a study conducted from 2008-2011 in a university hospital, 76% of *A. baumannii* isolates were resistant to meropenem and 79.5% of *A. baumannii* isolates were categorized as multi-drug resistant (Townsend et al., 2015). Although carbapenem resistance is quite common today, carbapenems are typically still considered the drug of choice. When carbapenem resistance is detected, treatment usually changes to include either sublactam or polymyxins, such as colistin (Peleg et al., 2008). However, resistance to each of these classes has also been reported. In fact, pan-drug resistant strains of *A. baumannii*, resistant to all current antibiotics, have also been reported (Peleg et al., 2008). If one becomes infected with a pan-drug resistant strain, no current antibiotic could be used for therapy.

While little clinical information exists about *A. baumannii* before the 1970s, it is widely assumed that the species was sensitive to most antibiotics. Because *Acinetobacter* infections were likely easily treatable, it was not regarded as a threat. Beginning in Europe in the early 1980s, documented hospital outbreaks of *A. baumannii* were described in England, France, Germany, Italy, Spain, and the Netherlands (Peleg et al., 2008). The subsequent spread *A.*

baumannii infection across the world is likely due to transportation and travel of colonized patients. In 1991 and 1992, outbreaks of carbapenem-resistant *A. baumannii* were reported from a New York City hospital; these strains were multi-drug resistant, only susceptible to polymyxins and ampicillin-sublactam. Since this time, this pathogen has been prominent in United States hospitals; the National Nosocomial Infection Surveillance system reported significant increases in resistance to amikacin, ceftazidime, and imipenem from 1986 to 2003 in the United States. However, the prominence of *A. baumannii* was especially noted beginning in March 2003, following the Iraq war. At this time, a number of soldiers suffered infected battle wounds due to this organism, leading to the nickname "Iraqibacter." This organism was later determined to have not been obtained from the environment or during battle, but rather from infected surfaces in field hospitals (Howard et al., 2012).

V. ANALYSIS OF *ACINETOBACTER BAUMANNII*

The relationship between bacteriophages and *Acinetobacter baumannii* has never been documented in scientific literature. Due to the correlation between phage elements and virulence—such as antibiotic resistance—in a number of other organisms, including *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella enterica,* and *Streptococcus pyogenes* (Chen et al., 2005; Fortier & Sekulovic, 2013; Hosseinidoust, van de Ven, & Tufenkji, 2013; Wagner & Waldor, 2002) I hypothesize that the acquisition of cryptic prophage elements within the *Acinetobacter baumannii* genome corresponds to the sudden increase in virulence and rapid evolution observed in this organism. Because *A. baumannii* is distinctly different from other species of *Acinetobacter*, which are not normally pathogenic, the acquisition and harboring of phage elements may correlate to the pathogenic potential of *A. baumannii* in contrast to non-*baumannii Acinetobacter* species. The inclusion of cryptic prophages within the genome throughout the history of *A. baumannii* may suggest that this species has undergone purifying selection in order to maintain and contribute to the rapid rise in virulence. Phage elements may also serve as active regulatory switches in *A. baumannii,* as they do in other species (Feiner et al., 2015).

a. Discovery of Novel Genetic Material

Two clinical isolates of *A. baumannii*, BR097 and BU310 were analyzed in order to better understand the pathogenicity of this organism. Both isolates were obtained from a single patient in 2012, although each is a different strain; BR097 was isolated from a respiratory specimen, while BU310 was isolated from urine. Because significant genotypic and phenotypic differences, it is assumed that each isolate was obtained independently of the other. The whole

genome shotgun sequencing projects for each specimen can be accessed through the NCBI database (Accession numbers NZ_JNFY00000000.1 and NZ_JNFZ00000000.1).

When *A. baumannii* strains BR097 and BU310 were first analyzed, the two strains were aligned against a reference strand of *A. baumannii* (ATCC 17978), isolated in 1951, to begin to analyze the differences in each. *A. baumannii* ATCC 17978 (Accession: NZ_CP018664.1) was previously completed to produce a whole chromosomal sequence, while the sequences of both BR097 and BU310 were incomplete. By aligning the contiguous segments of BR097 and BU310 to strain ATCC 17978, a draft quality chromosomal sequence was established for both strains. Alignment was achieved by using online bioinformatic software; both RAST: Rapid Annotation Using Subsystem Technology (Aziz et al., 2008; Brettin et al., 2015; Overbeek et al., 2014) and Mauve (A. C. E. Darling, Mau, Blattner, & Perna, 2004; A. E. Darling, Mau, & Perna, 2010) were utilized to establish alignment.

Upon alignment of these different isolates, a number of gaps were present within the chromosomal map (Figure 2). Such gaps represented DNA that was not contained in the reference *A. baumannii* ATCC 17978 genome. Indeed, this DNA was novel genetic information contained within the genomes of *A. baumannii* BR097 and BU310. Because it was not contained in the original reference strain of *A. baumannii* from 1951, it is likely that this information was either acquired through evolution prior to appearing in these two isolates or this information was acquired directly by these two isolates by mechanisms such as lateral gene transfer.

b. Identification of Phage Genes

These novel sequences were then annotated to determine where they originated and to understand the role they played in *A. baumannii.* A number of software programs were used to

identify code determining sequences and open reading frames contained within this DNA; those sequences that potentially coded for proteins were further analyzed to understand what kind of protein it codes for based on conserved domains, amino acid residues, transmembrane helices, and signal peptides. Software used included BLAST: Basic Local Alignment Search Tool, CDD: Conserved Domain Database Search, T-Coffee (Di Tommaso et al., 2011), WebLogo (Crooks, Hon, Chandonia, & Brenner, 2004), TMHMM: Transmembrane Helix Hidden Markov Models, SignalP, PSORT-b (Gardy et al., 2003), and Phobius (Kall, Krogh, & Sonnhammer, 2004).

After annotation and analysis, the majority of this novel DNA was determined to originate from bacteriophages and code for phage-related functions based on sequence homology and conserved domains. The PHAST (Zhou, Liang, Lynch, Dennis, & Wishart, 2011) and PHASTER (Arndt et al., 2016) prophage search tools were used to identify areas of intact, incomplete, and questionable prophage sequences from a library of prophage sequences. One intact prophage and a number of incomplete prophages were identified in both strains BR097 and BU310; they harbor an intact *Psychrobacter* Psymv2 phage as well as many incomplete *Acinetobacter* Bϕ-B1251 phages (Table 3 and 4).

Therefore, the acquisition of genetic information directly from bacteriophages has altered the genetic makeup of these strains. The prophage-imposed diversity among these three strains may represent clinically significant changes and account for the differences observed in *A. baumannii* today.

c. Analysis of Prophage Inclusion Among Other *Acinetobacter baumannii* Strains

In order to understand the evolutionary impact prophages may have on the development and expansion of antibiotic resistance in *A. baumannii*, other previously sequenced strains were analyzed to determine if they harbor prophage elements. The genomes of twenty-two clinical isolates of *Acinetobacter baumannii* were obtained from the public databases of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/genome/) (Table 2). These strains were chosen to represent a continuum of time, isolated beginning in 1950 through today. Together, these twenty-two strains represent a diverse group of isolates, differing in specimen source, geographical location, and antibiotic resistance patterns.

As before, the PHAST (Zhou et al., 2011) and PHASTER (Arndt et al., 2016) prophage search tools were used to identify areas of intact, incomplete, and questionable prophage sequences based on the number of genes present, the homology, and the function of the genes. Those marked as “incomplete” or “questionable” harbored genes that were identified in a specific phage, although not all of the genes of the bacteriophage are found within the bacterial host. To properly classify each region, a score is generated (guidelines established by phaster.ca); a score of 150 is considered a perfect score while scores of >90 are considered intact, 70-90 is considered “questionable,” and <70 is considered “incomplete” (Arndt et al., 2016; Zhou et al., 2011).

After analysis, it was found that all but one strain of *A. baumannii* contained two or more phage elements (Table 2). The one strain that did not contain phage genes, AYE, did acquire a resistance island that accounts for its virulence (Howard et al., 2012). Resistance islands are groups of resistance genes in a single segment of DNA that are passed horizontally between bacteria; these mobile elements are continuously lost and gained. The CIP70.10 strain, isolated

in 1950, contained two prophage elements (one intact and one incomplete) while the AB030 strain, isolated in 2014, contained fifteen prophage elements (four intact and eleven incomplete). There is obvious difference between these strains isolated 64 years apart. As anticipated, other *A. baumannii* strains isolated recently displayed similar patterns to our own isolates BR097 and BU310; all of these strains have seemed to maintain defective prophages, as well as intact prophages, within their genome.

With the exception of AYE, all other strains maintained at least one defective (incomplete) prophage within their genome (Tables 3 & 4). However, these strains were less likely to harbor intact prophages (Tables 3 & 4). Of the twenty-two isolates studied, six of the strains carried no intact prophages. Among the strains that did contain an intact prophage, the quantity of these phages was also significantly less than the number of incomplete prophage elements maintained. Most strains only carry one intact prophage while multiple incomplete elements are found in a single strain.

d. Relationship of Phage Domestication to Antibiotic Resistance and Virulence

Upon analyzing the placement of cryptic (incomplete) prophage elements within the genomes of *A. baumannii* BR097 and BU310, a phage replication initiation protein (Inoviridae protein Gp2) was identified that lied downstream from an RND multidrug efflux transporter and membrane fusion protein, as well as a transcriptional regulator (AraC) (Figure 2). In order to determine if phage genes had any effect on the antibiotic resistance potential within a strain, the same twenty-two isolates were also analyzed to identify antibiotic resistance genes.

The Comprehensive Antibiotic Resistance Database (CARD) (card.mcmaster.ca) was used to identify resistance genes by comparing the protein-coding sequences of an organism to

the database of sequenced resistance genes (Jia et al., 2017). This program is able to identify "perfect," "strict," and "loose" matches to known resistance genes. For this project, only "perfect" matches were searched in order to maintain reliable identification across all strains. Table 5 lists the resistance genes identified in each strain. These resistance genes contribute to resistance of different antibiotics through differing means; Table 6 summarizes the number of genes, the resulting resistance patterns, and the different mechanisms a strain may use to resist antibiotics.

e. Analysis of Prophage Inclusion in Other *Acinetobacter* Species

As previously described, *Acientobacter baumannii* is the only true pathogen within the genus *Acinetobacter.* With the exception of *A. baumannii*, other *Acinetobacter* species are ubiquitous in nature. Nonpathogenic species can be isolated from almost any environmental source, especially in soil, wastewater, and as human normal flora (Al Atrouni, Joly-Guillou, Hamze, & Kempf, 2016; Peleg et al., 2008). There are currently 48 species of *Acinetobacter* identified by the NCBI. *A. nosocomialis, A. pittii, A. ursingii, A. haemolyticus, A. lwoffii, A. parvus, A. junii* have been implicated in a few isolated human infections, although they are not typically considered human pathogens (Touchon et al., 2014).

The environment could potentially serve as a reservoir for antibiotic resistance in other *Acinetobacter* species. Non-*baumannii* strains isolated from hospital sewage, agricultural runoff, and polluted surface waters have been found to produce carbapenemase and extended-spectrum beta lactamase (ESBL) enzymes (Al Atrouni et al., 2016). Resistance genes are easily spread in the environment; *Acinetobacter* is particularly well-suited for the natural environment, as this

genus is able to resist desiccation and can survive in unfavorable, nutrient-limited conditions for long periods of time (Al Atrouni et al., 2016; Peleg et al., 2008).

If other *Acinetobacter* species are also capable of acquiring resistance mechanisms such as antibiotic-inactivating enzymes, why is *A. baumannii* the only true pathogen? In order to differentiate *A. baumannii* from other *Acinetobacter* species, other sequenced species of *Acinetobacter* were also analyzed to determine their prophage content (Table 9). The PHAST and PHASTER programs were utilized for this analysis. Due to their low degree of pathogenicity, fewer strains of other species have been sequenced and made available through the NCBI. Those that were available were analyzed, but represent an altered view of this population, as many of these strains were involved in rare human infections and have hence been studied.

After analysis, all but one strain (*A. calcoaceticus* PHEA-2) of non-*baumannii* *Acinetobacter* spp. harbored an intact or incomplete prophage. Unlike most strains of *A. baumannii,* these strains frequently only harbored a single phage that was often intact. The diversity of phages identified in these strains presented with a much greater variety than those of *A. baumannii* (Table 10). Phages identified from other *Acinetobacter* species included *Enterobacteria* phage mEp235, *Psychrobacter* phage pOW20, *Burkholderia* phage phiE255, *Pseudomonas* phage PhiCTX, *Haemophilus* phage SuMu, and many others. *Acinetobacter* phage Bϕ-B1251 was isolated in nine of the twenty-one other species analyzed, a much lower percentage than that of *A. baumannii*.

VI. DISCUSSION

i. Acquisition of Novel Genetic Material

This data indicates that *A. baumannii* has acquired a significant amount of novel genetic material and continues to evolve through the years. As previously determined by numerous studies, the genome of *A. baumannii* has a high degree of plasticity and is easily made competent (Imperi et al., 2011). Therefore, the genome of *A. baumannii* is expected to be in constant flux as genetic changes occur. However, elements that remain within the genome despite these perturbations likely are significant to the change in pathogenicity of this organism. In this study, numerous changes were observed among these strains of *A. baumannii*; most of these changes could not be tracked over time and were, therefore, temporary elements. Some of these changes included acquisition of resistance genes that were not observed in other populations of this organism, as well as loss of intact prophages. Despite the inherent variability among strains, patterns were identified that were consistent among strains and are, therefore, significant.

ii. Integration of Phage Elements

Indeed, prophages represent the largest reservoir of variability in *A. baumannii*. While a number of intact phages were identified, most of the phage genes that were discovered within the genome of *A. baumannii* were, however, not intact phages. As the ultimate goal of a prophage is lysis (extraction from the host genome), whether from lytic or lysogenic life cycles, the perpetuation of prophages within the host genome is not expected to occur. Upon lysis, prophage elements are typically rapidly excised from the host, leaving very little, if any, genetic material behind. When mutations or deletions occur to the phage and it is no longer capable of lysis, the remaining segments of the genome persist within the host genome. These defective

"cryptic" prophages were commonly observed in *A. baumannii*, suggesting previous infection with the same prophage.

When analyzing prophage elements, it was noted that *A. baumannii* strains BR097 and BU310 tended to especially preserve segments coding for structural phage components including those for major capsid, integrase, and tail proteins. These components are typically found on the ends of intact prophages, assisting the phage with integrating into and extracting itself from the host genome. This finding is consistent with other studies that identified that genes encoded in the internal parts of a prophage, such as packaging genes, are more commonly removed than other genes located on the outer edges of a prophage (Bobay et al., 2014). There is still no explanation as to why these genes are conserved as opposed to others; future studies are needed to elucidate the selective processes that occur to maintain these genes.

Such extraneous material within the genome of an organism was often believed to be a negative event, compromising host fitness. Regardless of the type or function of the conserved genes, it is known that the insertion of foreign DNA, specifically bacteriophage DNA, into the bacterial chromosome certainly impacts the regulation and, therefore, function of bacteria. Ron Feiner described the consequences of chromosomal disruption by bacteriophages in 2015; he suggests that insertions into essential regions for function or regulation would not be tolerated and would, therefore, not be passed along to other generations. However, these insertions may be tolerated in situations where the integration of the phage would be among genes that were non-essential or only required in specific conditions, such as virulence factors (Feiner et al., 2015).

In *A. baumannii* BR097, phage insertion points were primarily in regions of the genome rich in genes coding for antibiotic resistance, fimbiral adhesion, and pilus assembly proteins,

among others. While these genes certainly influence the virulence of this organism, these genes are not necessary for survival. Despite the disruptive connotation phage genes carry in these accounts, they may not only be merely tolerated, but they may actually prove advantageous for their host. The location of integration must certainly not interfere with viability, but as long as the host is able to maintain or have very minimal detriment to its overall fitness, maintaining a cryptic prophage does not harm a host. In fact, the interactions phage genes experience with non-essential genes, such as virulence factors, may actually promote the expression of these defenses.

Such observations have been noted in other pathogenic bacteria, including *Shigella dysenteriae, Salmonella typhimurium,* and *Salmonella typhi.* In *Shigella dysenteriae,* the shiga toxin genes are surrounded on each side by isolated phage genes. The same phenomenon also occurs surrounding the sopE2 (involved in invasion of human epithelial cells) gene in *S. typhimurium* and the sspH (promotes attachment to host cells) and pertussis-like toxin genes in *S. typhi* (Canchaya et al., 2004). Other studies also suggest that the integration of phages within genes and operons actually may function as regulatory switches (Feiner et al., 2015).

While further studies are indicated, numerous instances of phage proteins located next to transcriptional regulators and antibiotic resistance genes, such as RND multidrug efflux pumps were found within our two clinical isolates of *A. baumannii*, suggesting that the species *A. baumannii* may experience similar phage interactions. One example is displayed in Figure 1. In this instance, a gene coding for a phage replication initiation protein (Inoviridae Gp2) was found upstream from genes coding for RND multidrug efflux pumps as well as transcriptional regulators. The clustering of phage genes nearby host genes coding for virulence factors and regulatory functions is likely not random. I theorize that the integration of phage genes in these

areas alters the regulation and expression of virulence factors such as antibiotic efflux pumps. Although all strains of *A. baumannii* inherently contain efflux pumps, those that are the most virulent (i.e. antibiotic resistant), are more recent strains that have acquired more phage elements, especially incomplete elements. Because phage genes are commonly found nearby genes encoding virulence factors, it is likely that a relationship does exist.

iii. Prophage Inclusion in the *Acinetobacter baumannii* Population

Significant amounts of prophage content were also discovered in other global strains of *A. baumannii.* Among the twenty-two isolates of *A. baumannii* that were studied, notable differences in geographic location, date of collection, and type of infection exist. However, similarities and trends can be discerned within the whole population, despite the apparent variance.

Both intact and incomplete prophage elements were found in *A. baumannii* strains, although the inclusion of incomplete elements was much more pervasive; incomplete elements were found in greater numbers and incomplete elements were even discovered in genomes that lacked intact prophages (see LAC-4 and BJAB0868). Cryptic (incomplete) prophages were observed in all but one strain (AYE), as previously discussed. In the twenty-one strains containing cryptic prophages, all harbored at least one element originating from *Acinetobacter* phage Bϕ-B1251 (also referred to as YMC/09/02/B1251).

The consistency observed in carrying prophage elements derived from this specific phage suggests that *A. baumannii* may have a preference for maintaining it. This is likely not random; as other studies have suggested, such similarity often results from a single ancestral phage insertion or from multiple integrations of the same phage over time (Bobay et al., 2014). As

discussed earlier, it is particularly unlikely for an intact phage to persist within a genome for long periods of time, as they will eventually lyse and kill their host. Intact prophages are also subject to rapid degradation by the host with time. Once defective, the phage carcass remains within the host. Because the same defective prophage continues to be found across the majority of strains, beginning as early as 1951 and continuing through today, it is probable that some of these elements were maintained through replication and evolution. Since they are not lost from the genome through lysis, they are more likely to be maintained as long as they do not confer a considerable fitness burden for the cell.

iv. Relationship of Phage Domestication to Antibiotic Resistance and Virulence

Mechanisms of antibiotic resistance are not always straightforward and may require phenotypic testing to truly elucidate resistance profiles. Some bacteria are intrinsically resistant to certain antibiotics based on physical characteristics—due to differences such as thick peptidoglycan cell walls in Gram positive bacteria and lipopolysaccharide in Gram negative bacteria—they may have indirect means of resisting antibiotics (such as forming biofilms or decreasing the number of porins), or they may acquire mutations to existing resistance mechanisms or regulatory pathways (Adams, Chan, Molyneaux, & Bonomo, 2010; Hornsey et al., 2011; Munita & Arias, 2016).

Consequently, it is nearly impossible to interpret an organism’s resistance capability based solely on genomic studies. Phenotypic susceptibility studies are often necessary to determine a resistance profile, as performed by clinical microbiology laboratories. Therefore, analysis by the CARD list was not comprehensive and does not consider intrinsic resistance, secondary mechanisms, and altered proteins (such as pbps—penicillin binding proteins).

However, the resistance profiles obtained from the CARD database can still provide an adequate surmise toward the actual resistance profiles of each strain.

Overall, *A. baumannii* does continue to constantly acquire new resistance genes with time. The specific genes it acquires also changes with time, likely in response to changing recommended antibiotic regimens. Older resistance genes are sometimes lost or are replaced. One notable trend was the eventual elimination of adeR from the genome of *A. baumannii* isolates (Table 5). This gene regulated antibiotic resistance to tetracyclines and aminoglycoside antibiotics by regulating the adeABC efflux pump (CARD, Table 7)(Higgins, Schneiders, Hamprecht, & Seifert, 2010). adeR was commonly found in the *A. baumannii* genome from 1951-2004, but the last time it was seen in a representative *A. baumannii* genome was 2008 (Table 5). This change was actually due to a novel mutation within the adeR gene, impacting its regulation of the adeABC efflux pump and resulting in overexpression of adeABC and, therefore, increased resistance to tetracycline and aminoglycoside antibiotics (Higgins et al., 2010). Tigecycline is currently a popular choice in therapy against *A. baumannii*, but its effectiveness is now jeopardized because of this mutation.

This is just one example of the various mutations and mechanisms *A. baumannii* has acquired to become more virulent. Due to the complexity of genetic interactions and factors associated with antibiotic resistance, it is difficult to attribute the rise of antibiotic resistance in *A. baumannii* solely to the acquisition of cryptic prophage elements. However, antibiotic resistance continues to increase, paralleling the increase in cryptic prophage elements in *A. baumannii*. A connection between these two elements may, indeed, exist, but the exact mechanism is unable to be discerned at this time.

v. Prophage Inclusion in Other *Acinetobacter* species

Among the genus *Acinetobacter*, *A. baumannii* is the only true pathogen—but what accounts for this difference? While other species are capable of causing human infections, they predominantly remain susceptible to most antibiotics. Numerous studies have failed to understand why the lifestyle of *A. baumannii* is distinctly unique. A number of theories have been proposed, including unique gene clusters (including genes coding for integrases, type IV secretion systems, pilus biogenesis, iron uptake, and metabolism functions) (Peleg et al., 2008) as well as differences in transcriptional regulators (Adams et al., 2010). These propositions may be true, but it is possible that these differences may even have originated from phages. Bacteriophages are one of the largest vehicles of horizontal gene transfer and, by definition, introduce foreign DNA into organisms; it is not unlikely that this is how *A. baumannii* acquired these differences and, as a consequence, became more virulent.

Phage domestication by purifying selection certainly seems to occur in *A. baumannii*, but other scientists studying this organism have overlooked this basic concept. The implications of increasing phage content seem to have been previously dismissed by this scientific community.

VII. CONCLUSIONS

Our community is not immune to the effects of antibiotic resistance, including that caused by *Acinetobacter baumannii*. Everyone's life is impacted by this threat, whether evident or not; our healthcare bills continue to increase, infections become more devastating, treatments no longer exist, and we are left defenseless against an enemy we once easily controlled. *A. baumannii* has established its place in our community whether we recognize it or not. As seen in by the clinical data in Table 1, *A. baumannii* continues to cause a steady number of hospital infections. This organism will not be eradicated anytime soon. The data, however, does not reflect the changes this species has undergone; the strains we encounter today are often significantly more virulent, and, therefore, more deadly than those of previous years.

A. baumannii has undoubtedly experienced changes over time, most notably by acquisition of phage genes. Within the *A. baumannii* species, increasing numbers of prophage elements are acquired throughout its evolution. When the first strain of this organism, isolated in 1950, was sequenced, it contained only minimal prophage content. Significant differences are observed in the most recently isolated strains. The total amount of genetic material obtained from bacteriophages (Graph 1) continues to rise exponentially from 1950 to today. Similar patterns are seen in prophage content solely from defective (incomplete) prophages (Graph 2), as well as prophage content exclusively from *Acinetobacter* phage Bϕ-B1251 (Graph 3). These patterns are remarkably similar because of the total prophage content harbored in strains of *A. baumannii*, the majority of the content is due to incomplete prophages. Also, among the inclusion of cryptic prophages, the majority of these defective prophage remnants originated from *Acinetobacter* phage Bϕ-B1251. Relatively consistent numbers of intact prophages are maintained, as they are continually gained and removed from the organism's DNA. Therefore,

intact prophages have no true impact on the evolution of this species; prophages only impact *A. baumannii* once they have lost their function and become cryptic. Even when such mutations occur, *A. baumannii* still appears to maintain selective pressure over the specific elements it retains.

This single species is strikingly different than other species within the genus *Acinetobacter*. The most obvious difference between this true pathogen and the environmental species it is closely related to appears to be delineated by prophage content. While non-*baumannii* species certainly also harbor phages, as most bacteria do, these phages and phage conservation patterns differ from those of *A. baumannii.* This species has undoubtedly maintained a significant amount of defective prophages—an occurrence that is only occasionally observed in other *Acinetobacter* species. Whether *A. baumannii* is inherently more competent than other species, and thus is more prone to acquisition of foreign DNA, is not clear. Despite some minor variability, one common theme exists: *A. baumannii*, distinctively different from other species of *Acinetobacter*, maintains a preference for genes originating from *Acinetobacter* phage Bϕ-B1251. This particular phage is found in environmental strains as well, but not as significantly as *A. baumannii*. This phage may not be conserved or selected for in these species because it does not confer benefit. Alternatively, these species may first be selecting for the benefits this phage may bestow. Because non-*baumannii Acinetobacter* species are first being recognized as causative agents of human infection, it is possible that this trend is due to the recent acquisition of incomplete Bϕ-B1251 elements. However, many more strains must be sequenced in order to analyze this theory.

As evidenced by Tables 5-7, *A. baumannii* continues to acquire antibiotic resistance determinants and virulence factors. The changes in resistance profiles vary among strains, but

one pattern is consistent: this species is becoming more resistant every year. During this time, it continues to be exposed to other phages, but seems to maintain preference for the Bϕ-B1251 phage genome. Our data demonstrates that the maintenance of Bϕ-B1251 phage elements corresponds with *A. baumannii*'s growing antibiotic resistance patterns. The unique inclination towards maintaining Bϕ-B1251 phage elements differentiates *A. baumannii* from other species of *Acinetobacter* that only rarely cause human infections and do not possess the same degree of pathogenicity as *A. baumannii*. Therefore, this difference is truly noteworthy.

VII. FUTURE DIRECTIONS AND IMPLICATIONS

Antibiotic resistant bacteria are inescapable in healthcare, and much attention has been given towards the discovery of novel therapeutics to kill pathogenic bacteria. One popular alternative suggests using bacteriophages as predacious agents (Abedon, 2014). Bacteriophages only are capable of killing their bacterial host and pose no harm to humans, as opposed to many toxic antibiotics. Phage therapy has been shown to be promising for a number of infections, especially against biofilm-producing organisms that are difficult to treat using antibiotics (Semler, Lynch, & Dennis, 2011; Waters et al., 2017). In 2012, Drs. Jongsoo Jeon and Jae-won Kim published an article suggesting the use of *Acinetobacter* phage YMC/09/02/B1251 ABA AP (synonymous to phage Bϕ-B1251) as therapy for *A. baumannii* infections. They noted that this phage was successful in killing a strain of carbapenem-resistant *A. baumannii* and concluded that this phage would be useful in treating patients with sepsis due to multi-drug resistant *A. baumannii* (Jeon, Kim, Yong, Lee, & Chong, 2012).

However, because this phage was found to lie defective within the genomes of a majority of sequenced *A. baumannii* isolates, this bacteriophage does not always behave as we might expect. The mutations we observed in *A. baumannii* represent the consequences that may result when a phage is incapable of producing lysis. Rather than killing bacteria, prophages (once defective) may actually cause bacteria to become more virulent. Careful consideration must especially be given to the Bϕ-B1251 phage if used as therapy. This single phage, as opposed to numerous others known to infect *A. baumannii*, is often maintained by this organism.

The interactions between bacterial pathogens and bacteriophages are not as simple or straightforward as some may believe them to be. More respect must be shown towards bacteriophages and their important role in bacterial evolution. Deliberately introducing

pathogenic bacteria to bacteriophages within the human biome may not only be ineffective, but catastrophic.

Hope, however, should not be lost. Numerous alternatives to antibiotics are now being tested. These include initiatives such as utilization of the CRISPR/Cas9 system to resensitize bacteria to antibiotics, competition by predatory bacteria, exposure of pathogens to UV light, or even vaccination against anticipated bacterial threats. As explained above, just as caution must be exercised before accepting bacteriophages as a means of therapy, all of these new alternatives must be well understood before being implemented in healthcare. Just as the development of antibiotics had consequences we are facing today, other unforeseen consequences may result from the use of these new therapies. With careful study, scientific discourse, and the expanding pool of resources available today, a community of scientists will be able to elucidate the best future therapy. Studies such as ours are essential to provide more information about possible consequences as we proceed through this journey.

Table 1. Numbers and Types of Infections due to *Acinetobacter baumannii* by Year at a Single Hospital in Louisville, KY

	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**% in 2016**
Blood Cultures	13	10	17	15	12	15	16	9.6
Anaerobic	8	15	19	10	15	18	19	11.4
Body Fluids	12	3	3	1	0	1	0	
Bone	0	2	0	0	0	0	0	
Catheter tip	2	3	1	2	4	0	2	1.2
Cerebral Spinal Fluid	1	0	0	0	0	1	0	
Duodenal	0	0	0	0	0	1	0	
Eye/Ear	0	0	3	2	1	1	1	0.6
Genital	0	0	0	0	0	1	0	
Respiratory	37	95	66	52	34	29	43	25.9
Tissue	7	3	11	8	5	9	8	4.8
Urine	19	21	21	18	14	18	30	18
Wound	20	26	29	35	20	36	47	28.3
Total Number per year	**119**	**178**	**170**	**143**	**105**	**130**	**166**	

Table 2. Clinical isolates of *Acinetobacter baumannii* used for analysis

Date Isolated	Strain*	Source	Location
1950	CIP70.10	Skin	United States
1951	ATCC 17978	Cerebrospinal Fluid	France
1982	A1	Unknown	United Kingdom
1994	AB307-0294	Blood	Buffalo, New York
1/2/1997	LAC-4	Hospital Outbreak	Los Angeles, California
2001	AYE	Urine	France
2004	AB0057	Blood	Washington DC
2005	ACICU	Cerebrospinal Fluid	Rome, Italy
4/20/2006	MDR-ZJ06	Blood	Zhejiang, China
3/1/2007	BJAB0868	Ascites	Beijing, China
2007	TCDC-AB0715	Unknown	Taiwan
10/19/2008	D36	Hospital Outbreak	Sydney, Australia
2009	XH860	Unknown	Hangzhou, China
2010	XH857	Sputum	Guangdong, China
3/1/2010	XH858	Sputum	Zhejiang China
7/31/2011	AC29	Endotracheal Aspirate	Malaysia
7/14/2012	Ab04-mff	Blood	Edmonton, Canada
10/28/2012	BU310	Urine	Louisville, Kentucky
11/8/2012	BR097	Sputum	Louisville, Kentucky
2013	IOMTU433	Unknown	Nepal
5/29/2014	XH386	Sputum	Hangzhou, China
8/29/2014	AB030	Blood	Winnipeg, Canada

*Strains obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/genome/)

Table 3. Analysis of Prophage Elements within Bacterial Chromosomes[1] Among Strains

STRAIN	Total Phage Elements[2]	Intact *Acinetobacter* phage BϕB1251	Incomplete *Acinetobacter* phage BϕB1251	Total Intact Prophages	Total Incomplete Elements
CIP70.10	2	0	0	1	1
ATCC 17978	3	1	1	1	2
A1	4	2	1	2	2
AB307-0294	2	1	0	1	1
LAC-4	4	0	2	0	4
AYE	0	0	0	0	0
AB0057	6	1	3	1	5
ACICU	3	2	1	2	1
MDR-ZJ06	5	3	0	4	1
BJAB0868	3	0	2	0	3
D36	5	2	0	4	1
XH860	4	0	3	1	3
XH857	3	1	0	1	2
XH858	5	0	3	1	4
TCDC-AB0715	8	1	3	2	6
AC29	3	1	2	1	2
Ab04-mff	4	1	2	1	3
BU310	10	0	6	1	9
BR097	5	0	4	1	4
IOMTU433	5	2	1	2	3
XH386	5	1	1	3	2
AB030	15	2	3	4	11

[1] No plasmid data is included in analysis
[2] Number obtained from PHASTER

Table 4. Prophages Other than *Acinetobacter* phage Bϕ-B1251 Found

STRAIN	Other Intact Prophages	Other Cryptic Prophages
CIP70.10	Mannheimia phage vB MhM 3927AP2	Pseudomonas phage Pf3
ATCC 17978	Enterobacteria phage mEp235	
A1	Psychrobacter phage pOW20-A	
AB307-0294		Psychrobacter phage pOW20-A
LAC-4		Stx2-converting phage 1717 Pelagiphage HTVC010P
AYE		
AB0057		Cronobacter phage ENT39118 Acinetobacter phage AP22
ACICU		
MDR-ZJ06	Haemophilus phage SuMu	Enterobacteria phage SfV
BJAB0868		
D36	Mannheimia phage vB MhM 3927AP2 Psychrobacter phage Psymv2	Burkholderia phage phi6442
XH860	Enterobacteria phage mEp235	
XH857		Escherichia phage PA2 Enterobacteria phage P1
XH858	Mannheimia phage vB MhM 3927AP2	Escherichia phage PA2
TCDC-AB0715	Mannheimia phage vB MhM 3927AP2	Pseudomonas phage F116 Psychrobacter phage pOW20-A Salmonella phage F11SE
AC29		
Ab04-mff		Myxococcus phage Mx8
BU310	Psychrobacter phage Psymv2	Megavirus chiliensis Invertebrate iridescent virus 6 Faustovirus
BR097	Psychrobacter phage Psymv2	
IOMTU433		Acinetobacter phage AP22 Enterobacteria phage mEp235
XH386	Enterobacteria phage mEp235 Salmonella phage SEN34	Enterobacteria phage mEp235
AB030	Enterobacteria phage mEp235 Enterobacteria phage mEpx2	

Table 5. Resistance Genes Identified in *Acinetobacter baumannii* Strains by CARD*

1950	CIP70.10	adeK, mexT, OXA-64
1951	ATCC 17978	adeK, adeR
1982	A1	adeK, adeR, adeA, abeS, aadA, catI, mexT, OXA-69
1994	AB307-0294	adeK, adeI, adeA, abeS, mexT, OXA-69
1997	LAC-4	adeK, adeI, abeS, mexT, sul2, OXA-68, OXA-235
2001	AYE	adeK, adeI, adeR, adeA, abeS, aadA, arr-2, catI, dfrA10, mexT, OXA-10, OXA-69, APH (3')-la, VEB-1
2004	AB0057	adeK, adeR, adeA, abeS, aadA, catI, mexT, OXA-69, OXA-23, APH (3')-la, TEM-1
2005	ACICU	adeK, abeS, mexT, OXA-66, sul1
2006	MDR-ZJ06	aadA, calB6, OXA-23, OXA-66, sul1
2007	TCDC-AB0715	aadA, abeS, catB8, mexT, OXA-23, OXA-66, sul1, sul2, TEM-1, APH(3')-la
2007	BJAB0868	abeS, ADC-2, mexT, OXA-23, TEM-1
2008	D36	adeK, adeI, adeR, adeA, abeS, mexT, OXA-23, OXA-69
2009	XH860	adeK, adeI, abeS, mexT, OXA-23, OXA-66, APH(3')-1a
2010	XH857	abeS, aadA, armA, catB8, mexT, msrE, OXA-23. OXA-66, sul1, TEM-1, APH (3')-la
2010	XH858	adeK, abeS, mexT, OXA-23, OXA-68
2011	AC29	adeK, abeS, armA, mexT, msrE, OXA-66, APH (3')-la, TEM-1
2012	Ab04-mff	adeK, adeI, abeS, mexT, OXA-23, OXA-68, sul2
2012	BU310	abeS, mexT, OXA-95
2012	BR097	adeK, adeI abeS, aadA, armA, catB8, mexT, msrE, OXA-23, OXA-24, OXA-66, OXA-95, sul1, APH (3')-Ia, TEM-1
2013	IOMTU433	adeK, adeI, mexT, NDM-1, OXA-23, OXA-104, bleomycin resistance protein (BRP)
2014	XH386	adeI, abeS, aadA, armA, CATB8, mexT, msrE, OXA-23, OXA-66, sul1, TEM-1, APH(3')-la
2014	AB030	adeK, abeS, mexT, OXA-23, OXA-65, sul2, TEM-1

*CARD data was limited to perfect matches; strict and loose matches were not included in this analysis

Table 6. Antibiotic Resistance Profiles and Mechanisms Conferred by Resistance Genes

	AMINOCOUMARIN RESISTANCE	AMINOGLYCOSIDE RESISTANCE	BETA-LACTAM RESISTANCE	CHLORAMPHENICOL RESISTANCE	FLUORQUINOLONE RESISTANCE	GLYCOPEPTIDE RESISTANCE	LINCOSAMIDE RESISTANCE	MACROLIDE RESISTANCE	RIFAMPIN RESISTANCE	STREPTOGRAMIN RESISTANCE	SULFONAMIDE RESISTANCE	TETRACYCLINE RESISTANCE	TRIMETHOPRIM RESISTANCE		ANTIBIOTIC INACTIVATION ENZYME	ANTIBIOTIC TARGET MODIFYING ENZYME	ANTIBIOTIC TARGET REPLACEMENT PROTEIN	EFFLUX PUMP CONFERRING RESISTANCE	GENE MODULATING AX EFFLUX	GENE INVOLVED IN ANTIBIOTIC SEQUESTRATION
CIP70.10	1		2	2	2		1	1	1			1	2		1			2	1	
ATCC 17978	1		1	1	1		1	1	1			2	1					2	1	
A1	1	1	2	3	2		1	1	1			2	2		3			5	2	
AB307-0294	2		3	3	3		2	2	2			3	3		1			5	1	
LAC-4	2		4	3	3		2	2	2		1	2	3		2		1	4	1	
AYE	2	2	5	4	3		2	2	3			4	4		7		1	6	2	
AB0057	1	2	3	3	2		1	1	1			3	2		6			5	2	
ACICU	1		2	2	2		1	1	1		1	1	2		1		1	3	1	
MDR-ZJ06		1	2	1							1				4		1			
TCDC-AB0715		2	3	2	1						2		1		6		2	2	1	
BJAB0868			3	1	1								1		3			2	1	
D36	2		4	3	3		2	2	2			4	3		2			6	2	
XH860	2	1	5	3	3		2	2	2			2	3		4			4	1	
XH857		3	3	1							1				6	1	1	3	1	
XH858	1		3	2	2		1	1	1			1	2		2			3	1	
AC29	1	2	3	2	2		1	2	1	1		1	2		3	1		4	1	
Ab04-mff	2		4	3	3		2	2	2			2	3		2			4	1	

BU310																				
BR097		3	5	2							1				8	1	1	4	1	
IOMTU-433			3			1									3			3	1	1
XH386	1	1	4	3	2		1	2	1	1	1	1	2		6	1	1	4	1	
AB030			3								1				3		1	3	1	

Table 7. Descriptions of Resistance Genes Identified by CARD

Gene Name	Effects on Resistance	Description
aadA	antibiotic inactivation enzyme, aminoglycoside resistance	ANT(3")-Ia is an aminoglycoside nucleotidyltransferase gene encoded by plasmids, transposons, integrons in Enterobacteriaceae, A. baumannii, P. aeruginosa and Vibrio cholerae
abeS	efflux pump conferring resistance	AbeS in an efflux pump of the SMR family of transporters found in Acinetobacter baumannii.
ADC-2	beta-lactam resistance gene, antibiotic inactivation enzyme	ADC-25 is a beta-lactamase found in Acinetobacter baumannii.
adeA	efflux pump conferring resistance, tetracycline resistance gene	AdeA is the membrane fusion protein of the multidrug efflux complex AdeABC.
adeI	efflux pump conferring resistance, tetracycline resistance gene, fluorquinolone resistance gene, chloramphenicol resistace gene, trimethoprim resistance gene, beta-lactam resistance gene, macrolide resistance gen, aminocoumarin resistance gene, lincosamide resistance gene, rifampin resistance gene	AdeI is the membrane fusion protein of the AdeIJK multidrug efflux complex.
adeK	efflux pump conferring resistance, tetracycline resistance gene, fluorquinolone resistance gene, chloramphenicol resistace gene, trimethoprim resistance gene, beta-lactam resistance gene, macrolide resistance gen, aminocoumarin resistance gene, lincosamide resistance gene, rifampin resistance gene	AdeK is the outer membrane factor protein in the adeIJK multidrug efflux complex.
adeR	gene modulating antibiotic efflux, efflux pump conferring resistance, tetracycline resistance gene	AdeR is a positive regulator of AdeABC efflux system. AdeR inactivation leads to susceptibility to aminoglycoside antibiotics
APH(3')-1a	antibiotic inactivation enzyme, aminoglycoside resistance	APH(3')-Ia is a transposon-encoded aminoglycoside phosphotransferase in E. coli and S. enterica. It is identical at the protein sequence to APH(3')-Ic, an aminoglycoside phosphotransferase encoded by plasmids, transposons and genomic islands in K. pneumoniae, A. baumannii, S. marcescens, Corynebacterium spp., Photobacterium spp. and Citrobacter spp.
armA	aminoglycoside resistance gene, antibiotic target modifying enzyme	ArmA is a 16S rRNA methyltransferase that targets mature or nearly mature 30S subunits. It transfers a methyl group from S-adenosyl-L-methionine to N7-G1405 of the 16S rRNA, an aminoglycoside binding site.

arr-2	antibiotic inactivation enzyme, rifampin resistance gene	arr-2 is an integron-encoded ribosyltransferase found in Pseudomonas aeruginosa
BRP (bleomycin resistance protein)	gene involved in antibiotic sequestration, glycopeptide resistance gene	A diverse set of acidic proteins that are able to complex bleomycin and thus prevent bleomycin-induced DNA cleavage
catB6	antibiotic inactivation enzyme, chloramphenicol resistance	catB6 is a plasmid-encoded variant of the cat gene found in Pseudomonas aeruginosa
catB8	antibiotic inactivation enzyme, chloramphenicol resistance	catB8 is a plasmid or integron-encoded variant of the cat gene found in Klebsiella pneumoniae, Salmonella typhi and Pseudomonas aeruginosa
catI	antibiotic inactivation enzyme, chloramphenicol resistance	catI is a chromosome and transposon-encoded variant of the cat gene found in Escherichia coli and Acinetobacter baumannii
dfrA10	trimethoprim resistance, antibiotic target replacement protein	dfrA10 is an integron-encoded dihydrofolate reductase found in Klebsiella pneumoniae
mexT	efflux pump conferring antibiotic resistance, gene modulating antibiotic efflux	MexT is a LysR-type transcriptional activator that positively regulates the expression of MexEF-OprN and OprD.
msrE	efflux pump conferring resistance, macrolide resistance gene, streptogramin resistance gene	MsrE is an ABC-efflux pump expressed to Klebsiella pneumoniae that confers resistance to erythromycin and streptogramin B antibiotics. It is associated with plasmid DNA.
NDM-1	beta-lactam resistance gene, antibiotic inactivation enzyme, meropenem, imipenem, carbapenem, cephalosporin resistance	NDM-1 is a metallo-beta-lactamase isolated from Klebsielle pneumoniae with nearly complete resistance to all beta-lactam antibiotics
OXA-10	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-10 is a beta-lactamase found in Acinetobacter baumannii and P. aeruginosa
OXA-23	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-23 is a beta-lactamase found in A. baumannii
OXA-24	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-24 is a beta-lactamase found in A. baumannii and P. aeruginosa
OXA-64	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-64 is a beta-lactamase found in A. baumannii
OXA-66	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-66 is a beta-lactamase found in A. baumannii
OXA-68	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-68 is a beta-lactamase found in A. baumannii
OXA-69	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-69 is a beta-lactamase found in A. baumannii
OXA-95	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-95 is a beta-lactamase found in A. baumannii
OXA-104	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-104 is a beta-lactamase found in A. baumannii

OXA-235	beta-lactam resistance gene, antibiotic inactivation enzyme	OXA-235 is a beta-lactamase found in A. baumannii
sul1	antibiotic target replacement protein,sulfonamide resistance gene	Sul1 is a sulfonamide resistant dihydropteroate synthase of Gram-negative bacteria. It is linked to other resistance genes of class 1 integrons.
sul2	antibiotic target replacement protein,sulfonamide resistance gene	Sul2 is a sulfonamide resistant dihydropteroate synthase of Gram-negative bacteria, usually found on small plasmids.
TEM-1	beta-lactam resistance gene, antibiotic inactivation enzyme	TEM-1 is a broad-spectrum beta-lactamase found in many Gram-negative bacteria
VEB-1	beta-lactam resistance gene, antibiotic inactivation enzyme	VEB-1 is a beta-lactamase found in Escherichia coli and Pseudomonas aeruginosa

Table 8. Genomic Comparisons of Phages and Antibiotic Resistance Genes

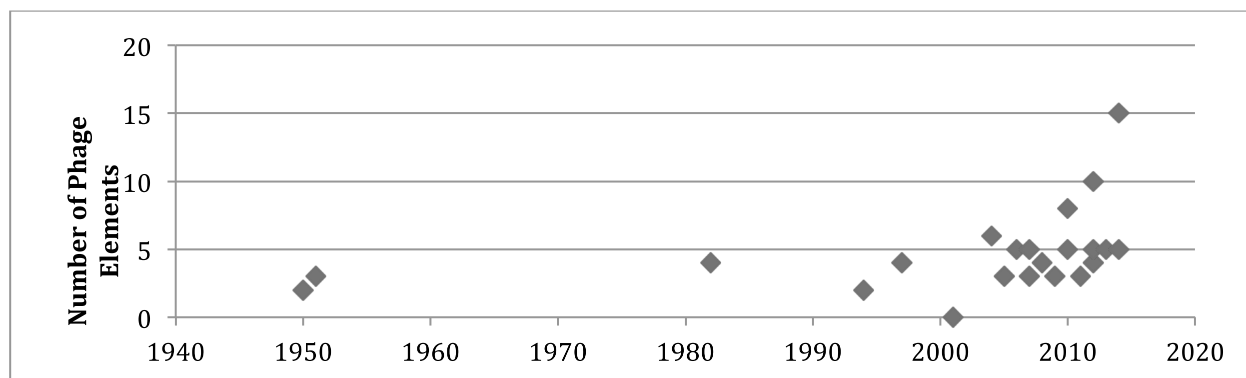
Year Collected	*A. baumannii* Strain	Number of Antibiotic Resistance Genes Identified	Number of Intact Phages	Number of Incomplete Prophages
1950	CIP70.10	3	1	1
1951	ATCC 17978	2	1	2
1982	A1	8	2	2
1994	AB307-0294	6	1	1
1997	LAC-4	7	0	4
2001	AYE	13	0	0
2004	AB0057	11	1	5
2005	ACICU	5	2	1
2006	MDR-ZJ06	5	4	1
2007	TCDC-AB0715	10	0	3
2007	BJAB0868	5	4	1
2008	D36	8	1	3
2009	XH860	7	1	2
2010	XH857	11	1	4
2010	XH858	5	2	6
2011	AC29	8	1	2
2012	Ab04-mff	7	1	3
2012	BU310	3	1	9
2012	BR097	15	1	4
2013	IOMTU433	7	2	3
2014	XH386	12	3	2
2014	AB030	7	4	11

Table 9. Isolates of Other *Acinetobacter* Species Used for Analysis

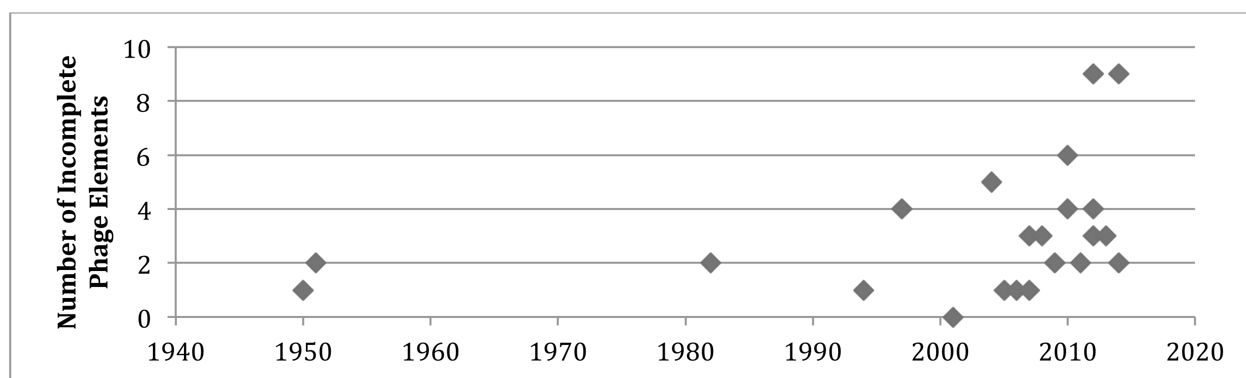
***Acinetobacter* Species**	**Strain**	**GenBank Accession Number**	**Source**
baylyi	ADP1	CR543861.1	Soil
baylyi	DSM 14961 (CIP 107474)	PRJNA183290	Activated sludge plant
calcoaceticus	PHEA-2	CP002177.1	Industry wastewater
calcoaceticus	RUH2202	PRJNA38337	Skin flora
gerneri	DSM 14967	PRJNA224116	Activated sludge plant
haemolyticus	ATCC 19194	SAMN01917633	Nose flora
haemolyticus	TJS01	CP018871.1	CLINICAL- respiratory
johnsonii	SH046	PRJNA38339	Skin flora
johnsonii	XBB1	CP010350.1	Hospital sewage
junii	65	CP019041.1	Limnetic water
junii	SH205	PRJNA38341	GI flora
lwoffii	SH145	SAMN02463731	Skin flora
nosocomialis	6411	CP010368.1	CLINICAL
oleivorans	DR1	NC_014259	Rice paddy soil
parvus	NIPH 1103	490813056	Skin flora
pittii	ATCC 19004 (CIP 70.29)		
pittii	AP 882	CP014477.1	CLINICAL-wound
radioresistens	SH164	PRJNA38345	GI flora
radioresistens	SK82	PRJNA34081	Skin flora
soli	GFJ2	CP016896.1	Industrial, soil
ursingii	NIPH 706	491140751	Blood

Table 10. Prophage Content Found in Other *Acinetobacter* Species

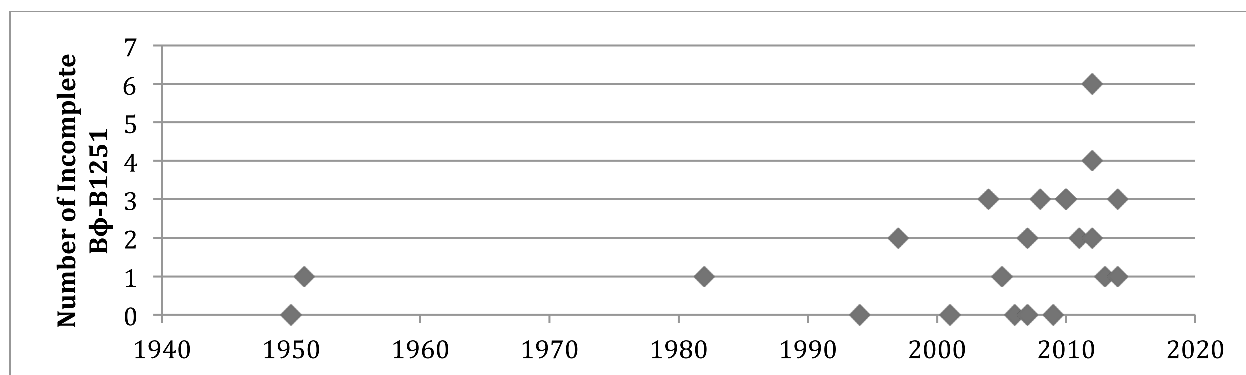
***Acinetobacter* Species**	Prophage Elements Identified by PHASTER	Intact Prophages	Incomplete Prophages	Questionable Prophages
baylyi	1		Psychro pOW20	
baylyi	1		Psychro pOW20	
calcoaceticus	0			
calcoaceticus	1			Acinet Bphi B1251
gerneri	1	Burkho phiE255		
haemolyticus	1	Acinet Bphi B1251		
haemolyticus	2	Pectob PP90	Enterofi AA91	
johnsonii	1	Entero mEp235		
johnsonii	4	Pseduo PhiCTX	Paenib tripp	Entero tyrion, Acinet Bphi B1251
junii	2	Entero mEp235		Paenib tripp
junii	3	Entero mEp235, Stx2 c 1717	Acinet Bphi B1251	
lwoffii	2	Shewan MR-1	Acinet Bphi B1251	
nosocomialis	1			Pseudo JBD93
oleivorans	2	Entero cdtl		Acinet Bphi B1251
parvus	1	Pseudo B3		
pittii	1		Burkho BcepMu	
pittii	1			Cronob vB GAP32
radioresistens	2		Acinet Bphi B1251	
radioresistens	5	Entero mEp235, Acinet Bphi B1251	Acinet Bphi B1251, Ostreo 2	
soli	4	Manhe vB MhM 3927AP2	Phage phiJL001	Acinet Bphi B1251
ursingii	1	Haemop SuMu		



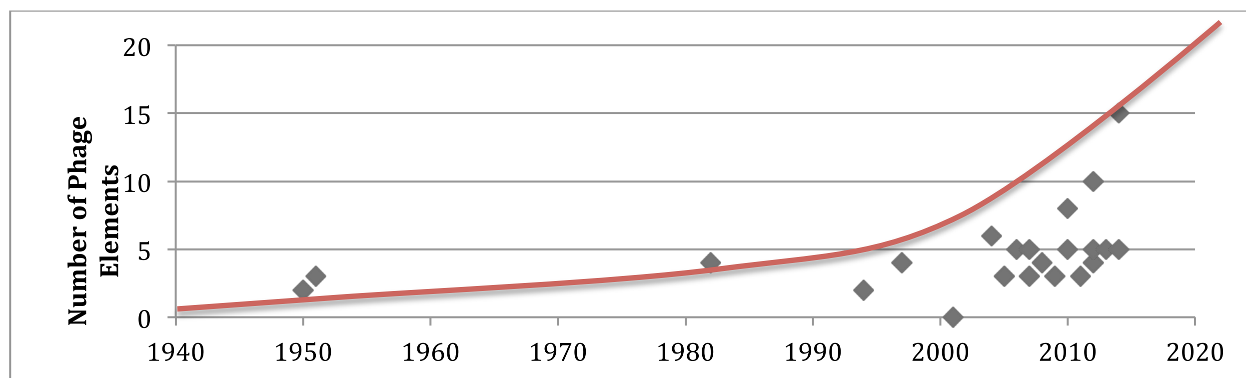
Graph 1. Total Phage Elements. *A. baumannii* strains isolated after 2000 appear to have steadily acquired more phage elements (both intact and incomplete prophages combined).



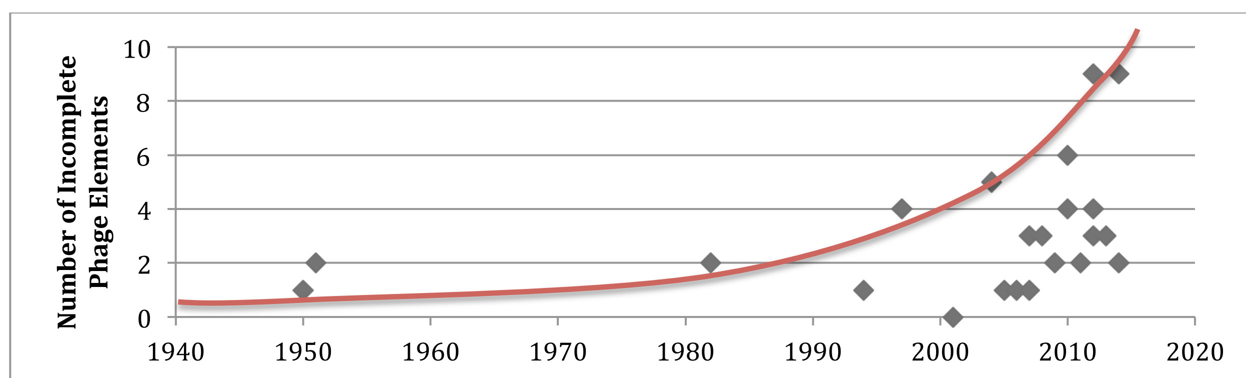
Graph 2. Number of Incomplete Phages. A steady rise in the number of incomplete prophage elements harbored by *A. baumannii* mirrors the rise in total phage elements (Graph 1). This rise in phage elements seems to parallel the rise in antibiotic resistance.



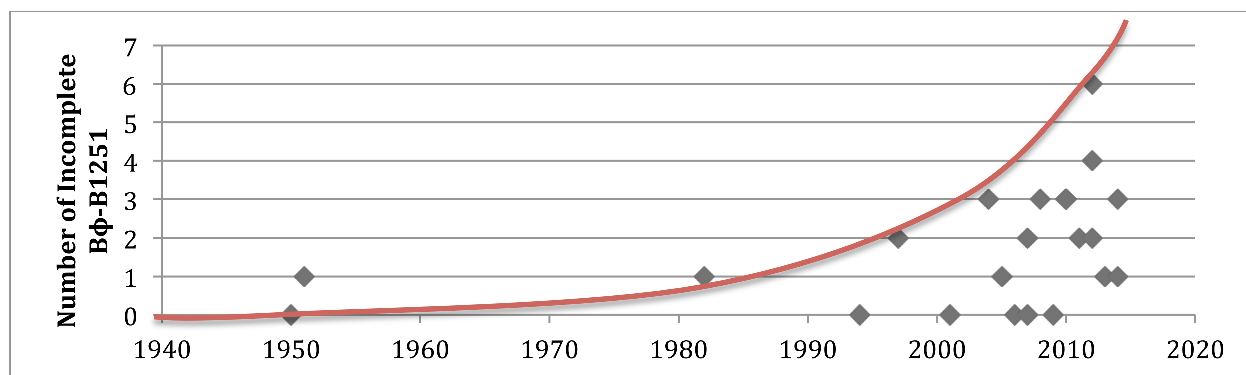
Graph 3. Number of Incomplete Bϕ-B1251. Compared to Graphs 1 and 2, the increase in both total phage elements and incomplete phages also mirrors the increase in incomplete Bϕ-B1251 elements. This single phage seems to be the most well-maintained phage element and is ubiquitous among a majority of *A. baumannii* strains.



Graph 4. **Total phage elements projected with exponential trendline**. Because different strains evolve at varying rates, only the most "evolved" strains were analyzed. Of these, a clear exponential pattern of phage acquisition (both intact and incomplete) was discerned



Graph 5. **Number of incomplete phages projected with exponential trendline**. Again, a clear exponential pattern of incomplete phage acquisition was discerned, suggesting that the evolution of *A. baumannii* in respect to prophage elements is due to incomplete, rather than intact, prophages.



Graph 6. **Number of incomplete Bϕ-B1251 phages projected with exponential trendline**. Finally, a clear exponential pattern of incomplete Bϕ-B1251 phage maintenance was also discerned. Therefore, of the incomplete phages contributing to *A. baumannii* diversity, *Acinetobacter* phage Bϕ-B1251 contributes the most to the exponential pattern we observe.

Figures

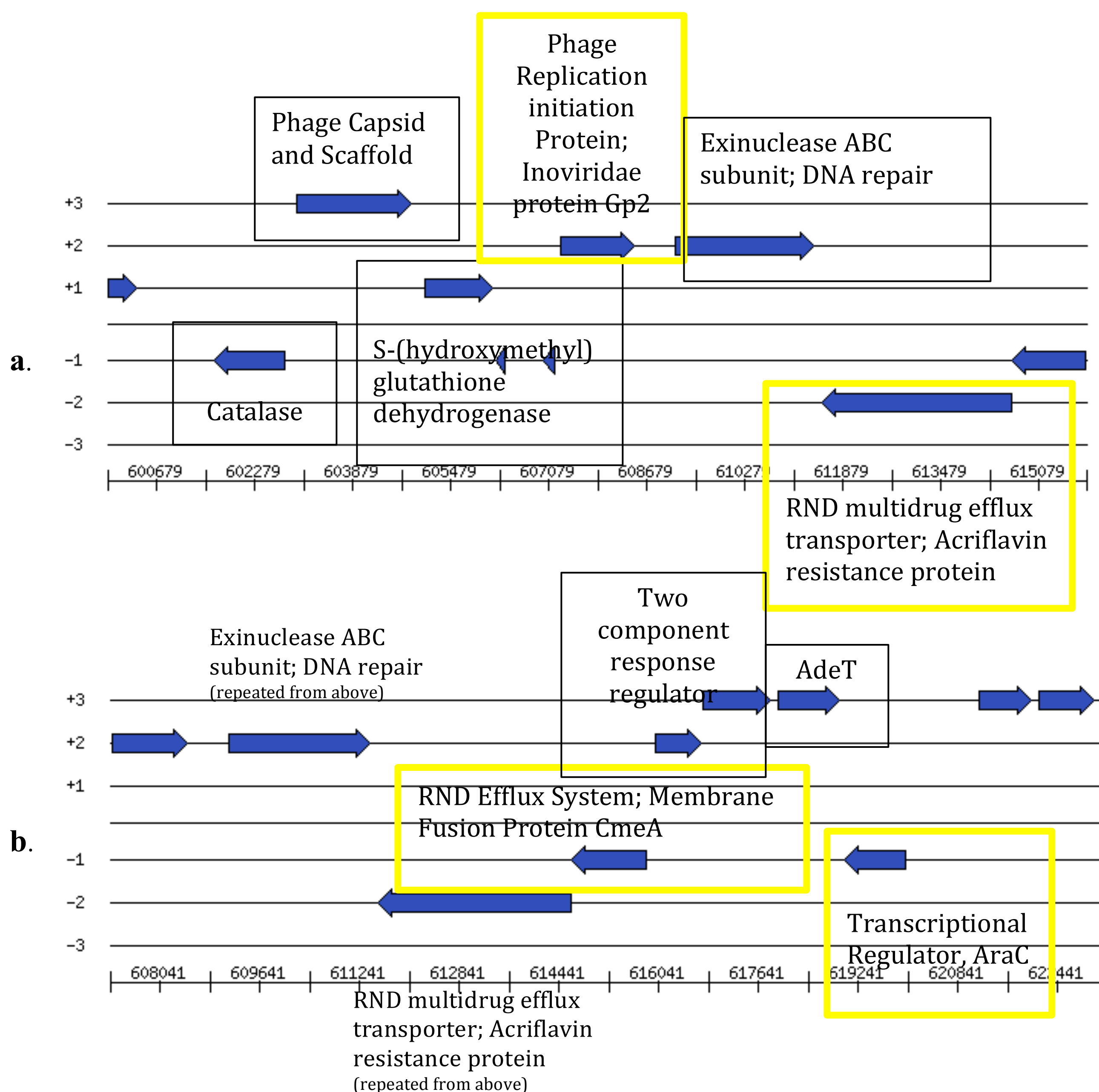


Figure 1. Placement of phage genes nearby antibiotic resistance genes and transcriptional regulators. Shown is contig 2 of *A. baumannii* BR097 bp 600679..622441 (begins at first line (a) and continues down to next line (b)). In this example, a phage replication initiation protein is located upstream from an RND multidrug efflux transporter and transcriptional regulator. Image generated using RAST SEEDviewer.

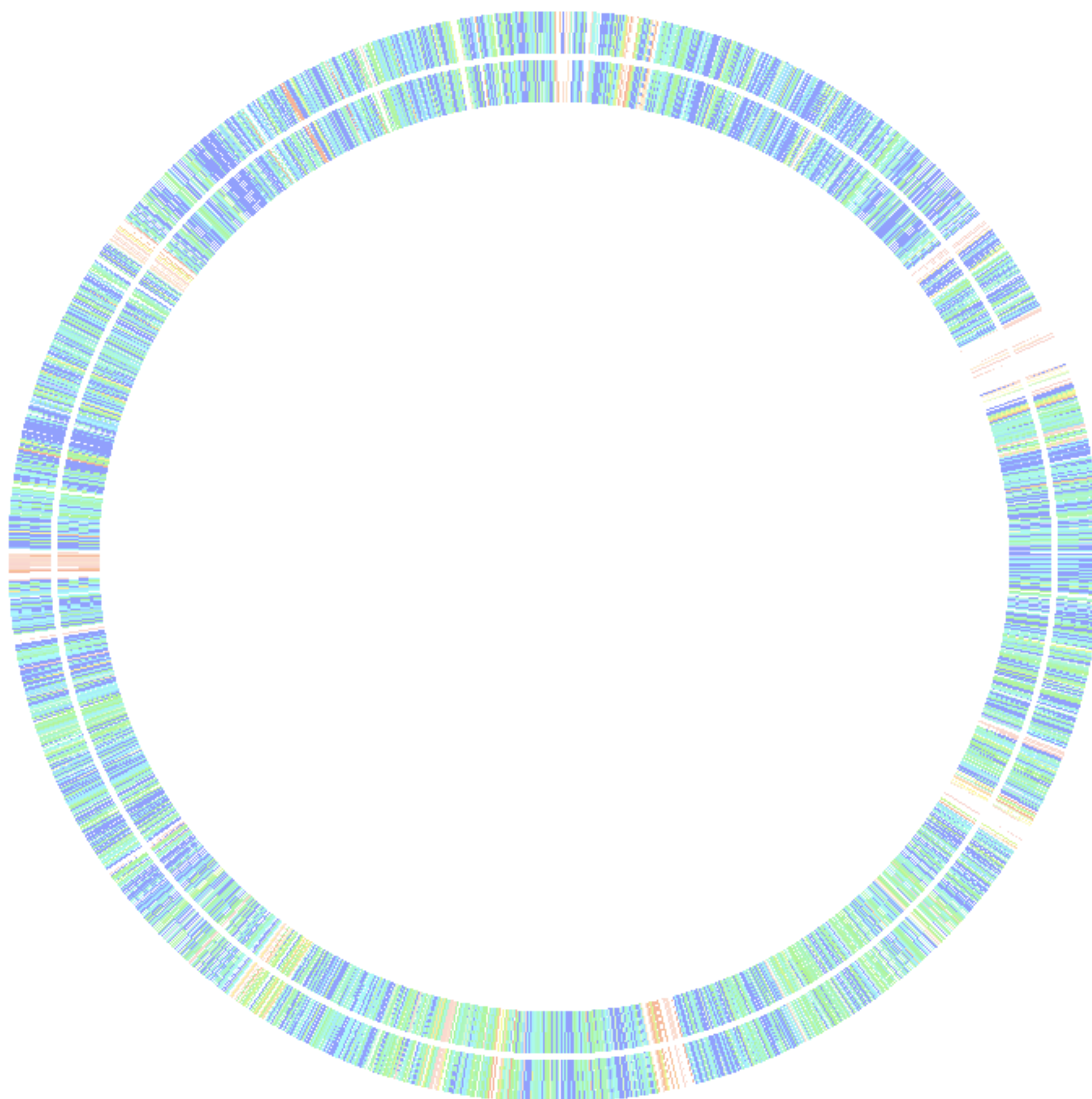


Figure 2. Genomic comparisons of *A. baumannii* BR097 and BU310 against reference strain *A. baumannii* ATCC 17978. The outermost ring represents BR097, while the inner ring represents the genome of BU310. The colors of each line within the ring (representing a gene within the genome) correspond to the percent of homology, ranging from dark blue (100%) to red. Areas of white represent novel genetic material that was not found within the reference genome and is, therefore, unique to the genomes used for comparison.

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